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Chromosomal Changes in the Chinese Hamster Following Skin Exposure to Tritiated Luminous Compounds

M. C. Colvin and J. M. Everts

Introduction

Work in this laboratory has demonstrated that tritium from tritiated luminous compounds is absorbed through the skin. The cytological damage caused by tritiated thymidine (^{3}HTdR) has been investigated by a number of workers. These experiments show that the effects observed include chromosome abnormalities with levels of about 1 μCi ^{3}H per gram of body mass.[1, 2] Early theoretical calculations[3, 4] suggested that ^{3}HTdR should be considered several orders of magnitude more toxic to mammalian systems than tritiated water.

This study investigates the absorption of tritium from tritiated luminous compounds through the skin of the Chinese hamster from an eight hour exposure and the resulting alterations to his chromosomes.

Two luminous compounds were selected for this study, one with a specific activity of 20 mCi/g and designated as RH, and the other designated MH with 200 mCi/g activity. Each hamster was exposed to 5 mg aliquots with the RH group receiving a dose of approximately 100 and the MH group receiving a dose of approximately 1000 μCi.

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Methods

Prior to the experiment, 30-day-old Chirese hamsters were placed in metabolic cages to become acclimated. One day pre-exposure the hair was clipped from the animals back, being careful not to cut the skin. The following morning 5 mg of the luminous compound was weighed into an area 1 cm^2 on a piece of plastic wrap mounted on

surgical tape. The bandage was carefully taped on the hamster's back by placing the animal upon the bandage with the compound facing the clipped area. An additional wider piece of tape was fastened around the animal's body to secure the inner tape and prevent loss of luminous compound.

Eight hours later the bandages were removed, and the remaining compound washed off carefully to avoid spreading over a larger area.

Urine samples were collected every 24 hours to monitor for tritium absorption and excretion. This was determined by counting an aliquot of urine in a liquid scintillation counter. The results were recorded as nanocuries of tritium per ml urine.

Control animals were handled in the same manner, but received no compound.

Seven and thirty days post exposure the hamsters were sacrificed for tissue culture and lung and kidney tissues were grown in Leighton tubes for chromosome studies. Replicates of each exposure period were run. The cells were allowed to grow for 72 hours, Colchemid added and allowed to act for 3 to 4 hours. This was followed by hypotonic treatment, fixation, and staining.

Results and Discussion

The stained slides were scanned under low power for metaphase cells in which the chromosomes were suitable for analysis. Metaphase cells that were spread and fixed in a manner to permit clear interpretation were then examined under phase oil immersion and classified as aberrant or non-aberrant cells.

A cell was considered as non-aberrant if there was no evidence

of chromosomal change. Aneuploid cells were not considered aberrant.

Metaphase cells containing chromosomes with structurial abnormalities were counted as aberrant. These changes were classified as chromatid and isolocus breaks, dicentrics, rings and exchanges. Chromosome or chromatid gaps were not scored as abnormalities, nor were fragments counted as chromosomes. Endoreduplications which occurred in 0.23% of total cells scored were classified as aberrant.

The majority of the aberrations were chromatid breaks with the frequency of other aberrations occurring in the following order, isolocus breaks, dicentrics, exchanges, endoreduplications and rings. This order held true for both the compounds, and at both exposure periods. However, there was some increase in the number of isolocus breaks at 30 days, but these did not outnumber the chromatid breaks. The results indicate that the most of the damage occurred during the G2 phase of the cell cycle. The slight increase in the frequency of isolocus breaks at 30 days could be due to second generation cells or to damage during the G1 phase of the cell cycle.

Totals of 15 and 16 animals were exposed to RII and MII compounds respectively. Figure 1 shows the daily urinary activity following cutaneous absorption of the two compounds divided by the administered dose. The curves are indicative of the relative absorption of the tritium activity from the two compounds. Urine samples collected from the control animals demonstrated levels of tritium on the order of 0.01 nCi per ml urine per hamster.

The chromosome damage to lung and kidney from the MH compound ranged from 5.39% at 7 days to 7.09% at 30 days. Exposure to the RH compound produced approximately the same percent of aberrations at both time periods (Table 1). As evidenced from Figure 1, more tritium activity was absorbed from the MH compound than from the RH compound. Since more activity was absorbed from the MH compound, one would expect to find a higher aberration percentage associated with it. This was not the case. Possibly the tritium from the RH compound was contained in such a form that it produced more damage. This aspect was not investigated further.

The standard deviation was calculated by assuming a binomial distribution. For such a distribution $\sigma = \sqrt{Npq}$ and for the percentage $\left(\frac{\sigma}{N}\right) = \sqrt{\frac{pq}{N}}$. The error presented in Table 1 is $\pm 2\sigma$. From these data it is apparent that there is a significant difference between the controls and the test animals. The test animals have an aberration number 15 to 20 standard deviations greater than the controls.

Conclusions

The present study has investigated the possibility of chromosomal damage following skin absorption of tritium from luminous compounds in the Chinese hamster. The following conclusions were reached:

1. Tritium was absorbed through the skin of Chinese hamsters as indicated by the appearance of significant amounts of tritium in the urine of the test animals. There was only a slight indication of any tritium in the urine of the control animals.

2. Chromosomal aberrations were produced at 7 and 30 days to both lung and kidney tissues. With both compounds tested, the percentage of lung and kidney cells containing aberrations was several times greater than the control values.
3. The type of aberrations most often found demonstrated that more damage occurred during the G2 phase of the cell cycle.

References

1. Bond, V. P., and L. E. Feinendegen, Health Physics 12:1007, 1966.
2. Feinendegen, L. E., Tritium Labeled Molecules in Biology and Medicine. Academic Press (N. Y.) 1967.
3. Cuild, W. R., Science 128:1308, 1958.
4. Oliver, R. and L. G. Lajtha, Nature, London 185:91, 1960.

Table 1. Effect of Two Tritiated Luminous Compounds on the Percent of Aberrant Cells in Adult Hamster 7 and 30 Days After Exposure

		7 days			30 days		
Activity	Tissue	Total Cells Scored	Aberrant	Percent Aberrant	Total Cells Scored	Aberrant	Percent Aberrant
MH Administered dose 993 μCi per hamster	Kidney	408	22	5.39 ± 2.24	406	25	6.15 ± 2.38
	Lung	643	39	6.06 ± 1.88	987	70	7.09 ± 1.64
TH Administered dose 95 μCi per hamster	Kidney	975	75	7.69 ± 1.70	421	30	7.12 ± 2.50
	Lung	466	33	7.08 ± 2.42	624	42	6.73 ± 2.00
Controls	Kidney	761	7	0.91 ± 0.68	918	4	0.43 ± 0.44
	Lung	501	4	0.79 ± 0.80	1297	17	1.31 ± 0.64

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